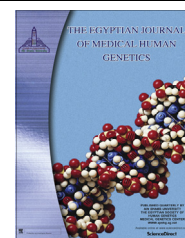




Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com


ORIGINAL ARTICLE

Circulating cell free DNA as a predictor of systemic lupus erythematosus severity and monitoring of therapy



Olfat M. Hendy ^{a,*}, Tawfik Abdel Motalib ^b, Mona A. El Shafie ^c,
 Fatma A. Khalaf ^d, Sobhy E. Kotb ^e, Aziza Khalil ^f, Salwa R. Ali ^g

^a Department of Clinical Pathology, National Liver Institute, Menoufiya University, Egypt

^b Department of Microbiology, National Liver Institute, Menoufiya University, Egypt

^c Department of Clinical Pathology, Menoufiya Faculty of Medicine, Egypt

^d Department of Clinical Biochemistry, National Liver Institute, Menoufiya University, Egypt

^e Department of Pediatric, Dubai Hospital, United Arab Emirates

^f Department of Physiology, Al Azhar Faculty of Medicine (for Girls), Egypt

^g Department of Internal Medicine, Al Azhar Faculty of Medicine (for Girls), Egypt

Received 10 June 2015; accepted 1 July 2015

Available online 29 July 2015

KEYWORDS

SLE;
 cf-DNA;
 Disease severity;
 C3;
 C4;
 CRP;
 Procalcitonin;
 RT-PCR;
 Anti-nucleosome

Abstract *Background:* Systemic lupus erythematosus (SLE) is the most heterogeneous chronic autoimmune disease; it is characterized by the presence of auto reactive B and T cells, responsible for the aberrant production of a broad and heterogeneous group of autoantibodies. Recent studies using various detection methods have demonstrated the elevations of circulating DNA in SLE patients.

Aim of the study: The current study aimed to measure cell-free DNA (cf-DNA) in SLE patients as a potential tool to predict disease activity and treatment follow up.

Subjects and methods: 52 of SLE patients with age ranging from 10 to 48 years were randomly selected and 25 healthy subjects with age and gender matched with the patients were included as a control group. Thorough clinical examination stressing on the central nervous system, vascular, renal, rash, musculoskeletal, mucocutaneous manifestations, and fever was done for patients. The following investigations were done: Complete blood count (CBC), kidney function tests, C-reactive protein (CRP), routine autoantibodies for autoimmune diseases, complements (C3 & C4), anti-nucleosome antibodies and cf-DNA by real time PCR (RT-PCR).

Results: The levels of anti-double stranded DNA (anti-dsDNA), anti-nucleosome Ab, and cf-DNA were significantly increased in SLE patients compared to controls. The cf-DNA level was correlated to markers of disease severity namely CRP and anti-nucleosome. A significant reduction in levels of cf-DNA, anti-nucleosome Ab and anti-dsDNA was noticed after therapy.

* Corresponding author at: National Liver Institute, Menoufiya University, Egypt. Mobile: +20 01000217406.

E-mail address: olfat_hendy@hotmail.com (O.M. Hendy).

Peer review under responsibility of Ain Shams University.

<http://dx.doi.org/10.1016/j.ejmhg.2015.07.001>

1110-8630 © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of Ain Shams University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conclusion: Our findings support that the measurement of cf-DNA appears to be a useful marker in addition to laboratory tests used in SLE diagnosis. High correlation with markers of disease severity suggesting its role in disease pathogenesis and decreasing its level after therapy makes it to be a marker of treatment follow-up.

© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of Ain Shams University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Circulating cell-free DNA (cf-DNA) has been found in the plasma of human subjects. It has been extensively studied over the past few decades. Supported by theory and observation, two major sources of cf-DNA have been postulated: first, fragmented DNA released as a consequence of cell death (apoptosis/necrosis of blood and tissue cells) and, second, active metabolic secretion of DNA from cells. Considerable research efforts had been made on the use of cf-DNA as a biomarker in cancer diagnosis [1].

In various pathologic conditions, qualitative and quantitative changes in circulating DNA have also been shown, such as mutations, deletions, methylations and microsatellite aberrations which are distinct from those in benign conditions, and thus may be useful in the diagnosis of cancer [2]. Only small amounts of serum or plasma DNA have been observed in healthy individuals, whereas high concentrations had been described in patients with various malignancies and in those with several benign diseases, such as infections, sepsis, trauma, stroke, and autoimmune diseases [3,4]. Because most of these disorders are associated with increased rates of cell death events, from apoptosis or necrosis, these mechanisms are considered to be the main sources for circulating DNA. Active release of DNA by lymphocytes is thought to be of minor relevance [4,6].

For many years, free DNA research has been focused on examining the level of free DNA in autoimmune diseases like rheumatoid arthritis [7], systemic sclerosis [8] and primary Sjogren's syndrome [9]. In case of rheumatoid arthritis, Leon et al. [7] had discovered higher concentrations of free DNA in both plasma and synovial fluid than in healthy subjects while the increasing intensity was correlated with the symptom severity and the level of the tissue damage. Unlike them, Mosca et al. [8] did not establish the significant difference in cf-DNA concentration in patients with systemic sclerosis and in healthy subjects, but based on the cf-DNA level they could make a difference between patients with active disease and those with the inactive one.

Systemic lupus erythematosus (SLE) is an autoimmune disease that has the potential of affecting multiple organ systems, including the skin, muscles, bones, lungs, kidneys, as well as the cardiovascular and central nervous systems [10,11].

SLE can cause various tissue inflammation and damage in a chronic manner and cell death [12]. Cell death has been regarded as an important event in the pathogenesis of SLE, as it leads to the release of antigens, such as nucleic acids, for immune complex formation, and that DNA-antibody complexes in the circulation are one of the hallmarks of SLE. DNA-antibody complexes may trigger a cascade of immune responses against the bodily tissues of the SLE patients [13,14].

SLE was one of the pathological conditions reported to be associated with the presence of circulating DNA nearly 59 years ago [15]. Since then, studies using various detection methods have demonstrated the elevations of circulating DNA in SLE patients [16,17]. Investigating whether and to what degree fluctuations in cf-DNA levels in patients with SLE might correspond to disease severity was the goal of many investigations [8,17]. The most recent data seem to exclude measuring cf-DNA as an inexpensive, simple and quick tool to assess disease activity in patients with SLE [9,17].

The application of molecular biologic techniques has allowed the molecular characterization of cf-DNA in certain pathologic and physiologic conditions. Various methods have been established for the measurement of circulating DNA. Quantification of DNA in plasma and serum by real-time PCR is widely accepted as standard and detects all kinds of free and protein bound circulating DNA [5]. However, there have been very few studies reporting the detailed biological characterization of circulating DNA in SLE [18]. The extremely variable clinical manifestations and the absence of effective tests to monitor disease activity present a challenge for clinical management.

1.1. Aim of the study

The current study aimed to measure the circulating cell free DNA (cf-DNA) as a potential tool to predict disease severity and treatment follow up in patients with SLE.

2. Patient and methods

Fifty-two of SLE patients attending the Menoufiya and Al Azhar University Hospitals between February 2013 and September 2014 were included in the study. Samples were taken from patients who fulfilled at least four of the American College of Rheumatology criteria for the diagnosis of SLE [19]. They were 40 females and 12 males with age ranging from 10–48 years, mean \pm SD (28.42 ± 11.43). Twenty-five healthy subjects (18 female and 7 males) age and gender matched with the patients were included in the study as a control group.

All patients were subjected to: full history and thorough clinical examination stressing on the central nervous system, vascular, renal, rash, musculoskeletal, mucocutaneous manifestations, and fever. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was calculated for all patients, a score of 8 or more was defined as active disease at the beginning of the study [20]. Fourteen patients had SLEDAI score of 6 or less and hence were considered inactive SLE; the remaining 38 were categorized to have active SLE disease.

2.1. Exclusion criteria

Patients with any concurrent disease, positive viral markers, malignant tumors, history of previous blood transfusion, other autoimmune diseases, primary anti-phospho-lipid syndrome, chronic or acute inflammatory diseases were excluded from the study.

3. Therapy follow-up

Cytotoxic drugs; cyclophosphamide, azathioprine, or mycophenolate mofetil were given to SLE patients according to the therapy protocol. In 38 of the 52 SLE patients after therapy follow-up, blood was collected at an interval of 1–13 months and re-analyzed in the same way. All study procedures were approved by the Ethics Committee of the faculty college of Medicine. All subjects were informed orally about the procedures and the aim of the study and gave written consent to participate. The work has been carried out in accordance with the World Medical Association (declaration of Helsinki) for experiments in humans.

3.1. Blood sampling

Ten ml of blood was collected under complete aseptic conditions, and divided into 3 vacutainer tubes: 2 ml on EDTA-anticoagulated blood for complete blood count, 3.2 ml on plain tube for serology, auto-antibody determination, 1.8 ml on 3.2% of Na citrate for Lupus anticoagulant. The remaining 3 mL was put on EDTA-anti-coagulated blood for plasma separation by centrifugation of fresh EDTA blood at -4°C at 1600g for 10 min. The plasma fraction was transferred carefully and centrifuged in a second step at -4°C and 1600g for 5 min to remove any cellular debris. The supernatants were stored at -20°C until used for cf-DNA measurement.

Routine laboratory investigations were done, as Complete blood count (CBC) on Sysmex-KN21 automatic cell counter (Japan), kidney function tests on Integra 400 (Roche-Diagnostics, Germany) and CRP on Elecyses-20 (Roche-Diagnostic, Germany).

Antinuclear antibody (ANA) was measured by indirect immunofluorescence assay (IIF) on mouse kidney and stomach slides (Immco-Diagnostics, USA). Detection of auto-antibodies of SSA (Ro), SSB (La), anti-Sm and anti-RNP was done by ELISA semi-quantitative technique using INOVA Diagnostics (San Diego-USA). Anti-double stranded DNA (anti-dsDNA) titer was done by ELISA technique using INOVA Diagnostics (San Diego-USA).

Anti-Cardiolipin antibodies (APL IgM & APL IgG) were detected by APhL[®] IgG and IgM ELISA Kit (Louisville diagnostics-USA). Lupus anticoagulant was done by the ACTICLOT[®] diluted partial thrombin time (dPTT) test (AMERICAN DIAGNOSTICA GmbH). It is used for the qualitative diagnostic determination of LA in patient plasmas.

Determination of complement C3 and C4 levels in serum was done on automated Elecyses-20 (Roche-Diagnostic, Germany).

Anti-nucleosome antibodies' (IgG) detection was done by ELISA kit (D-TEK, Belgium, Germany). Principle: briefly, after incubation of diluted serum sample in the micro-well coated with purified nucleosomes. If anti-nucleosome antibody

was present in the serum, it binds to specific antigens. Unbound or excess antibodies were removed by washing, and HPR-conjugate rabbit antibodies against human IgG were added to wells to form antigen antibody complexes. After incubation and second wash to remove excess conjugate, the TMB/substrate solution was added. If the enzyme activity was present, it generates blue reaction which is measured colorimetric by an ELISA plate reader at 450 nm. The absorbance was directly proportional to anti-nucleosome antibody concentration in serum sample.

3.2. Detection of plasma cell free DNA (cf-DNA) by real time PCR

DNA was extracted from 400 μl plasma using QIA amp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The final elution was performed in 50 μl of AE buffer of the QIA amp DNA blood mini kit, and the eluted DNA was stored at -20°C until further processing.

For analyzing cf-DNA, the GAPDH housekeeping gene has been used with forward 5'-CCCCACACACATGCACTT-ACC-3' and reverse 5'-CCTAGTCCCAGGGCTTTGATT-3' primers, and 5'-MGB-TAGGAAGGACAGGCAAC-VIC-3' as the probe [21] has been applied. Five ml of DNA elution was used as a template for the real-time PCR analysis. The PCR was performed using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, ABI). The real-time PCR was carried out in 25 μl of total reaction volume containing 5 μl of DNA, 12.5 μl of TaqMan[®] Universal PCR Master Mix, four primers, and two probes using two minute incubation at 50°C . The reaction was processed by an initial denaturation step at 95°C for 10 min and 40 cycles of 1 min at 60°C and 15 s at 95°C . For the multiplex TaqMan amplification of the concentration of primers and probes was optimized. The optimal concentration of the primers and probes for duplex real-time PCR is 0.6 μM for each primer and 0.4 μM for each probe [22]. The positive reaction was detected by the accumulation of a fluorescent signal. The cycles required for the fluorescent signal to cross the threshold are defined as cycle threshold (CT).

The CT values can also be converted into quantities according to standard curves generated by dilution of human genomic DNA with a known concentration for generating the standard curves as follows: Each reaction included a calibration curve of 6.6 ng to 6.6 pg genomic DNA made from 4 serial dilutions of purchased human genomic DNA (0.2 g/L in 10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0), (Roche Diagnostics GmbH-Germany) plus 2 known dilutions (50 and 500 pg DNA) used as controls in all experiments [23,24].

3.3. Statistical analysis

All mean values were expressed as mean \pm standard deviation (SD). The distribution of each continuous variable was examined statistically for normality. Variables normally distributed were compared using Student *t*-test. In contrast, variables not normally distributed were compared using the Mann-Whitney test. Spearman correlation test was used to assess the

relationship between variables in the same group. Statistical significance of the recorded OR, a two-sided $p < 0.05$ was considered to be significant. All statistical analyses were performed using Computer program SPSS version 20.

4. Results

Female predominance was evident as expected, with a female/male ratio of 3.3:1. The mean age for all patients' was 28.42 years. The mean illness duration was 8.32 years. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was 7.31. The frequency of clinical manifestations was photosensitivity (67.3%), arthralgia/myalgia (59.6%), renal manifestations (46.2%), cutaneous manifestations (40.4%), arthritis (32.6%) and serositis (11.5%), as percentage of total patients. Raynaud's phenomenon (9.6%), neurological manifestations (9.6%), and hematological manifestations (7.69%) were the least common clinical manifestations.

The main autoantibodies detected were ANA (96.2%), anti-dsDNA (80.8%), anti-Ro (48.1%), anti-RNP (32.7%), and anti-La (26.9%). Anti-Cardiolipin IgM (21.2%) anti-Cardiolipin IgG (15.4%) and lupus anticoagulants (13.5%) were the least detected antibodies (Table 1).

While comparing the demographic data of patient and control groups, there was no statistical significant difference regarding age and sex (p value > 0.05) for both. In contrast, the laboratory investigations showed that, the hemoglobin (Hb) concentration, total leukocytic counts (TLC), platelets and C3 were significantly low in SLE patients compared to health controls (p value < 0.05 , < 0.05 , < 0.01) respectively, while, ESR, CRP, anti-dsDNA, anti-nucleosome Ab, and cf-DNA were significantly higher in SLE patients compared to controls (p values were < 0.001 , < 0.01 , < 0.05 , < 0.001 ,

Table 1 Characteristic data of the SLE patients.

Characteristics of SLE patients ($N = 52$)	
Female/male (ratio)	40/12 (3.3:1)
Age (years)	28.42 \pm 11.43
Disease duration/years (M \pm SD)	8.32 \pm 6.14
SLEDAI (M \pm SD)	7.31 \pm 5.26
Clinical Manifestations:	Number (%)
• Arthralgia/myalgia	31 (59.6%)
• Arthritis	17 (32.6%)
• Renal manifestations	24 (46.2%)
• Hematological manifestations	4 (7.69%)
• Neurological manifestations	5 (9.6%)
• Cutaneous manifestations	21 (40.4%)
• Photosensitivity	35 (67.3%)
• Serositis	6 (11.5%)
• Raynaud's phenomenon	5 (9.6%)
Auto-antibodies:	Number (%)
• Anti-dsDNA	42 (80.8%)
• ANA	50 (96.2%)
• Anti-Ro	25 (48.1%)
• Anti-La	14 (26.9%)
• Anti-Sm	10 (19.2%)
• Anti-RNP	17 (32.7%)
• Anti-Cardiolipin IgM	11 (21.2%)
• Anti-Cardiolipin IgG	8 (15.4%)
• Lupus anticoagulant	7 (13.5%)

Table 2 Comparison between the laboratory data of patients and controls.

Parameters	SLE Patients ($n = 52$) (M \pm SD)	Control group ($n = 25$) (M \pm SD)	p value
Age (years)	28.42 \pm 11.43	32.58 \pm 14.22	> 0.05
Female (No. %)	40 (76.9%)	18 (72%)	> 0.05
ESR (mm/h)	91.42 \pm 34.8	11.23 \pm 6.75	< 0.001
Hb (g/dL)	10.53 \pm 1.72	13.25 \pm 1.18	< 0.05
TLC ($\times 10^9/L$)	6.22 \pm 3.14	9.54 \pm 3.65	< 0.05
Platelet count ($\times 10^9/L$)	131.2 \pm 54.1	276.1 \pm 41.72	< 0.01
CRP (mg/L)	9.53 \pm 6.72	0.28 \pm 0.03	< 0.01
C3 (mg/dl)	48.22 \pm 11.03	88.61 \pm 9.11	< 0.01
C4 (mg/dl)	19.32 \pm 4.71	26.81 \pm 7.65	> 0.05
Anti-dsDNA (IU/ml)	38.52 \pm 7.24	2.73 \pm 1.51	< 0.001
Anti-nucleosome Ab (IU/ml)	56.41 \pm 29.26	9.81 \pm 2.37	< 0.001
cf-DNA (ng/mL)	16.31 \pm 2.58	5.47 \pm 1.94	< 0.01

< 0.001 and < 0.01) respectively. The C4 levels were decreased, but did not reach statistical difference (Table 2).

Regarding the correlation study, there was a significant negative correlation between cf-DNA levels and ANA, anti-dsDNA level, and C3 ($r = -0.45$, $p < 0.05$; $r = -0.38$, $p < 0.05$; $r = -0.57$, $p < 0.01$) respectively. In contrast a positive correlation was found between cf-DNA level and CRP, anti-nucleosome Ab serum level ($r = 0.41$, $p < 0.05$; $r = 0.36$, $p < 0.05$) respectively. No correlation was found between cf-DNA level and each of age, gender, SLEDAI score or C4 ($p > 0.05$ for each) (Table 4).

Comparison between the laboratory data of SLE patients before and after therapy revealed a statistically significant reduction in antibodies after therapy. Anti-nucleosome Ab was reduced from a pretreatment mean of 56.41 IU/ml to a post treatment mean of 21.56 IU/ml (p value < 0.05). Anti-dsDNA was reduced from a pretreatment mean of 38.52 IU/ml to a post treatment mean of 13.25 IU/ml (p value < 0.05). Cf-DNA was reduced from a pretreatment mean of 16.31 ng/mL to a post treatment mean of 7.15 ng/mL (p value < 0.01) (Table 3 and Fig. 1).

Table 3 Correlation of cf-DNA level to patient's data ($n = 52$).

Parameters	cf-DNA		
	r	p value	Sign. value
Age	0.07	> 0.05	NS
Gender	0.13	> 0.5	NS
SLEDAI score	-0.19	> 0.05	NS
ANA	-0.45	< 0.05	S
Anti-dsDNA	-0.38	< 0.05	S
CRP	0.41	< 0.05	S
C3	-0.57	< 0.01	HS
C4	0.13	> 0.05	NS
Anti-nucleosome Ab	0.36	< 0.05	S

Table 4 Comparison between the laboratory data of SLE patients before and after treatment.

Parameters	SLE Patients before therapy (<i>n</i> = 52) (M ± SD)	After therapy (<i>n</i> = 38) (M ± SD)	<i>p</i> value
Anti-nucleosome Ab (IU/ml)	56.41 ± 29.26	21.56 ± 7.42	<0.05
Anti-dsDNA (IU/ml)	38.52 ± 7.24	13.25 ± 2.14	<0.05
cf-DNA (ng/mL)	16.31 ± 2.58	7.15 ± 2.11	<0.01

5. Discussion

The fluctuations in cell free DNA (cf-DNA) levels in patients with SLE which might correspond to disease severity were the goal of many investigations. This study was aimed to analyze cf-DNA in a group of SLE patients with different disease status and have clinical manifestations, to assess the fluctuation of cf-DNA level in relation to the course of the disease progression and medical treatment.

In the present study, the serum level of cf-DNA was significantly higher in SLE patients than in the healthy controls. This result was in accordance with the studies carried out by Atamaniuk et al. [17], Breetbach et al. [25], Suzan et al. [26]. The higher level of cf-DNA in SLE patients could result from ineffective clearance of apoptotic and necrotic cells [27], the release of DNA from neutrophils extracellular traps and its impaired degradation was also described in SLE [28]. Additionally, the neutrophil extracellular traps are implicated in sterile inflammation and could contribute to auto-inflammatory conditions, vascular inflammation and atherogenesis [13].

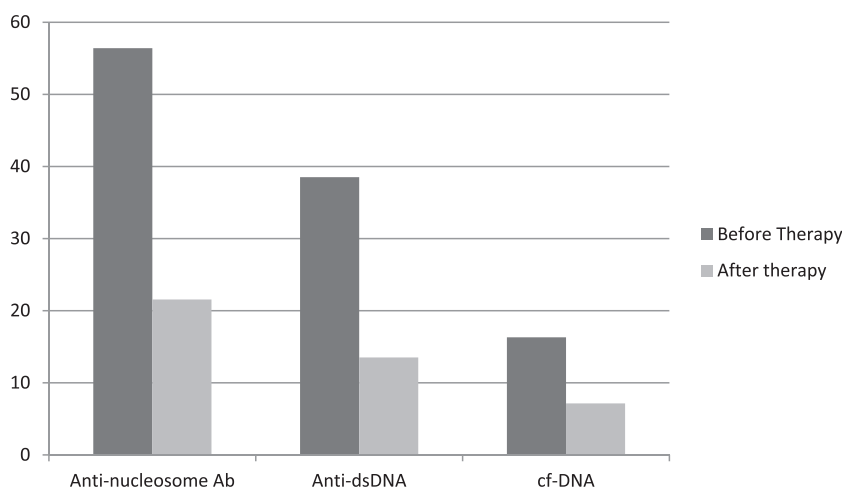
The serum level of C3 was significantly lower in our SLE patients than in the controls and in spite of C4 decrease in SLE patients, it did not reach a statistical significance. SLE is usually associated with complement consumption, resulting in low serum levels of C3 and C4 levels. This may be due to a concomitant phenomenon involved in the generation and increase of cf-DNA [13].

In an attempt to associate between cf-DNA concentrations and complement components in SLE, we observed that, although no correlation was found between cf-DNA and C4, there is an inverse correlation between the cf-DNA level and C3, in SLE patients. This result is not surprising since SLE associated complement consumption, resulting in low levels of C3 and C4, could be a concomitant phenomenon involved in the generation and increase of cfDNA [13].

In contrast, Suzan et al. [26] found that the complement factors C3 and C4 correlated positively with cfDNA concentrations in both study populations. Barteloni et al. [9] did not find any correlation between cf-DNA and complement components C3 and C4. It was also reported that fluctuations in complement and anti-dsDNA-antibodies did not predict disease flares which led the authors to the conclusion that alternative biomarkers should be tested. There was a good rationale to use complement activation products as a marker for disease activity. However, available studies have conflicting results, showing correlation of complement component and the disease activity in some but not in other studies. Some of this may result from methodological differences, such as the use of plasma versus serum and the differences of disease activity [29].

A positive correlation of cfDNA with CRP in our SLE patients could be a further hint regarding the involvement of cfDNA in inflammatory responses. In contrast, Suzan et al. [26], found no relation between cf-DNA and CRP levels in SLE patients, and explained that it could be due to the fact that the serum level of CRP was hardly changed during SLE flare [13]. Therefore, a simultaneous rise of cf-DNA and CRP in the case of high disease activity could be expected.

In the current study, anti-dsDNA level was significantly higher in SLE patients than in controls. Increasing lines of evidence suggested that dsDNA plays an important role in the pathogenesis of SLE as it can cause inflammatory responses [30]. Additionally, anti-dsDNA was negatively correlated significantly with the serum level of cf-DNA in the studied SLE patients. This was in agreement with the result of Isenberg et al. [31] study, who mentioned a well-documented phenomenon is that titer of anti dsDNA antibodies dropped in flare up of SLE disease [32]. Also, Macanovic et al. [33] reported that, the development of nephritic syndrome in some

**Figure 1** Mean plasma levels of cf-DNA in SLE patients before and after treatment.

SLE patients is accompanied by low levels of anti-dsDNA. These results could be explained by the fact that accelerated tissue deposition during active state leads to the decrease of Ab titer in the circulation.

In our study, there was a negative correlation between cf-DNA and ANA levels in SLE patients. This could be explained by the possibility that the high amount of antibody bound nucleic acid could impede the detection of cf-DNA in the circulation either by the formation of complexes or by clearance from the circulation [34].

Moreover, in the current study, the anti-nucleosome Ab was significantly higher in SLE patients compared to controls, and its level is positively correlated with cf-DNA. This finding is in agreement with that of Amoura and Piette [35], who reported the prominence of nucleosomes which circulate at high levels in some autoimmune diseases such as SLE; it has been speculated that highly accelerated rates of apoptosis, and/or abnormal sites or abnormal processing of apoptotic cells could lead to autoantibody production. Also, nucleosomes may elicit the production of interleukin-6 and stimulation of lympho-proliferation and IgG synthesis by splenic B cells. This could result in a polyclonal activation that triggers both a specific (nucleosome driven) and nonspecific antibody production [36].

In attempts to assess fluctuation of the level of cf-DNA during the course of SLE, we compared the serum level of cf-DNA in relation to SELADI score. There was no significant correlation observed between the serum level of cf-DNA and SELADI score in our SLE patients. This goes in accordance with the results of the study carried out by Barteloni et al. [9] and Suzan et al. [26], who stated that, no correlation was detected between the serum level of cf-DNA and SELADI score in their studies. Based on their finding, Atamaniuk et al. [17] excluded that cf-DNA is a suitable marker for SLE activity. However, the SELADI is used as a common activity marker for SLE but it is also known that this parameter does not necessarily reflect the severity of disease activity. SELADI score only rates the presence or absence of items and not the grade of severity in most of its items [37]. Hence, the lack of correlation is not surprising.

Moreover, the medical evaluation of disease usually consists of a combination of SELADI score, patient symptoms, laboratory parameters, and a significant correlation with cf-DNA. Furthermore, a significant reduction of the serum levels of cf-DNA, anti-dsDNA, and anti-nucleosome after specific drugs therapy for SLE disease indicates the prognostic value of cf-DNA in SLE disease.

6. Conclusion

The cf-DNA levels were significantly higher in SLE patients compared to healthy controls. The positive relationship between its level and markers of diseases severity may suggest its role in disease pathogenesis and decreasing its level after therapy make it to be a possible marker of treatment follow-up. Further studies on a larger patient population are still needed to confirm these results.

Conflict of interest

None declared.

References

- [1] Mittra I, Nair NK, Mishra PK. Nucleic acids in circulation: are they harmful to the host? *J Biosci* 2012;37:301–12.
- [2] Rainer TH, Wong LKS, Lam W, Yuen E, Lam NYL, Metrewel C, et al. Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. *Clin Chem* 2003;49: 562–9.
- [3] Sozzi G, Conte D, Leon M, Ciricione R, Roz L, Ratcliffe C, et al. Quantification of free circulating DNA as a diagnostic marker in lung cancer. *J Clin Oncol* 2003;21:3902–8.
- [4] Zeerleder S, Zwart B, Willemin WA, Aarden LA, Groeneveld AB, Caliezi C, et al. Elevated nucleosome levels in systemic inflammation and sepsis. *Crit Care Med* 2003;31:1947–51.
- [5] Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitation's and evidence for their origin from apoptotic and necrotic cells. *Cancer Res* 2001;61:1659–965.
- [6] Holdenrieder S, Stieber P. Apoptotic markers in cancer. *Clin Biochem* 2004;37:605–17.
- [7] Leon SA, Ehrlich GE, Shapiro B, Labbate VA. Free DNA in the serum of rheumatoid arthritis patients. *J Rheumatol* 1977;4: 139–43.
- [8] Mosca M, Giuliano T, Cuomo G, Doveri M, Tani C, Curcio M, et al. Cell-free DNA in the plasma of patients with systemic sclerosis. *Clin Rheumatol* 2009;28:1437–40.
- [9] Barteloni E, Ludovini V, Alunno A, Pistola L, Bistoni O, Crino L, et al. Increased levels of circulating DNA in patients with systemic autoimmune diseases: A possible marker of disease activity in Sjogren's syndrome. *Lupus* 2011;20:928–35.
- [10] Gladman DD, Urowitz MB. Prognosis, mortality and morbidity in systemic lupus erythematosus. In: Wallace DJ, Hahn BH, editors. *Dubois' lupus erythematosus*. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1333–53 [7th ed.].
- [11] Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011; 365(22):2110–21.
- [12] Cervera R. European working party on systemic lupus erythematosus morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore)* 2003;82(5):299–308.
- [13] Pisetsky DS. The immune response to cell death in SLE. *Autoimmune Rev* 2004;3(7–8):500–4.
- [14] Su KY, Pisetsky DS. The role of extracellular DNA in autoimmunity in SLE. *Scand J Immunol* 2009;70(3):175–83.
- [15] Tan EM, Schur PH, Carr RI, Kunkel HG. Deoxy-nucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J Clin Invest* 1966;45(11): 1732–40.
- [16] Chen JA. Sensitive detection of plasma/serum DNA in patients with systemic lupus erythematosus. *Autoimmunity* 2007;40(4): 307–10.
- [17] Atamaniuk J, Hsiak YY, Mustak M, Bernhar Erlacher L, Fodinger M. Analyzing cell-free plasma DNA and SLE disease activity. *Eur J Clin Invest* 2011;41:579–83.
- [18] Salamunic IL. Laboratory diagnosis of autoimmune diseases new technologies, old dilemmas. *Biochem Med* 2010;20:45–56.
- [19] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725–8.
- [20] Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI: a disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;35:630–40.
- [21] Walker JA, Hedges DJ, Perodeau BP, Landry KE, Stoilova N, Laborde ME, et al. Multiplex polymerase chain reaction for simultaneous quantitation of human nuclear, mitochondrial, and

- male Y-chromosome DNA: application in human identification. *Anal Biochem* 2005;337:89–97.
- [22] Fan AX, Radpour R, Haghighi MM, Kohler C, Xia P, Hahn S, et al. Mitochondrial DNA content in paired normal and cancerous breast tissue samples from patients with breast cancer. *J Cancer Res* 2009;5:463–6.
- [23] Atamaniuk J, Ruzicka K, Stuhlmeier KM, Karimi A, Eigner M, Mueller MM. Cell-free plasma DNA: a marker for apoptosis during hemodialysis. *Clin Chem* 2006;52:523–6.
- [24] Xia P, Radpour R, Zachariah R, Fan AX, Kohler C, Hahn S, et al. Simultaneous quantitative assessment of circulating cell-free mitochondrial and nuclear DNA by multiplex real-time PCR. *Genet Mol Biol* 2009;32:20–4.
- [25] Brechtbach S, Tug S, Simon P. Circulating cell-free DNA: an upcoming molecular marker in exercise physiology. *Sports Med* 2012;42:565–86.
- [26] Suzan T, Suzan H, Julia M, Daniela Z, Thomas K, Andreas S, et al. Correlation between cell free DNA levels and medical evaluation of disease progression in systemic lupus erythematosus patients. *Cell Immunol* 2014;292:32–9.
- [27] Munoz LE, Gaip US, Franz S, Sheriff A, Voll RE, Kalden JR, et al. SLE – a disease of clearance deficiency. *Rheumatology (Oxford)* 2005;44:1101–7.
- [28] Hakkim A, Fürnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A* 2010;107:9813–8.
- [29] Gillet Gabor, Edward T, Larissa L, Lipsky Peter E. Biomarkers in systemic lupus erythematosus. *Arthritis Rheum* 2004;50(7): 2048–65.
- [30] Frese S, Diamond B. Structural modification of DNA– a therapeutic option in SLE? *Nat Rev Rheumatol* 2011;7:733–8. <http://dx.doi.org/10.1038/nrrheum.2011.153>.
- [31] Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-dsDNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007;46:1052–6.
- [32] Magder LS, Ho A, Barr SG, Petri M. Decreases in anti-double-stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. *Arthritis Rheum* 2001;44:2342–9.
- [33] Macanovic M, Hogarth MB, Lachmann PJ. Anti-DNA antibodies in the urine of lupus nephritis patients. *Nephrol Dial Transplant* 1999;14(6):1418–24.
- [34] Amoura Z, Piette J, Chabre H, Cacoub P, Papo T, Wechsler B, et al. Circulating plasma levels of nucleosomes in correlation with serum anti-nucleosome antibody titers and absence of clear association. *Arthritis Rheum* 1997;40:2217–25.
- [35] Amoura Z, Piette JC. Role of the nucleosome in the physiopathology of systemic lupus erythematosus. *Ann Med Interne (Paris)* 2003;154:25–32.
- [36] Amoura Z, Koutouzov S, Chabre H, Cacoub P, Amoura I, Musset L, et al. Presence of anti-nucleosome antibodies in a restricted set of connective tissue diseases: anti-nucleosome antibodies of IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. *Arthritis Rheum* 2000;43(76–84): 38.
- [37] Mirzayan MJ, Schmidt RE, Witte T. Prognostic parameters for flare in systemic lupus erythematosus. *Rheumatology (Oxford)* 2000;39:1316–9.